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Bioaccumulation of metals (Cd, Cu, Ni, Pb and Zn) in suspended cultures of blue mussels exposed to different environmental conditions

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ABSTRACT

Farming of suspended mussels is important for generating high protein food and animal feed or for removing nutrients in eutrophic systems. However, the harvested mussels must not be severely contaminated by pollutants posing a potential health risk for the consumers. The present study estimated the bioaccumulation of cadmium, copper, nickel, lead and zinc in suspended blue mussels (*Mytilus edulis* L.) in the Limfjorden, Denmark, based on observations and modelling. Modelling was used to assess the suitability of suspended blue mussels as animal feed and food products at sea water metal concentrations corresponding to Good Ecological Status (GES) in the European Union Water Framework Directive (WFD) and in future climate change scenarios (higher metal concentrations and higher temperatures). For this purpose, GES is interpreted as good chemical status for the metals using the Environmental Quality Standards (EQS) defined in the WFD priority substance daughter directives. Observations showed that suspended mussels were healthy with respect to metal pollution and generally less polluted than benthic mussels due to the smaller contact with the contaminated sediment. The model results showed that the WFD targets for Cd, Ni and Pb are not protective with respect to marine mussel production and probably should be reduced for marine waters. Climate changes may increase the metal contamination of mussels, but not to any critical level at the relatively unpolluted study sites. In conclusion, WFD targets should be revised to assure that the corresponding body burdens of metals in mussels are below the safety limits according to the EU Directives and the Norwegian classification for animal feed and food production.

1. Introduction

Aquaculture becomes more important as the demand for food increases worldwide (Duarte et al. 2009). The production of bivalves is the second most important type of aquaculture and has increased worldwide by 6.2% per year during the past decade (Duarte et al. 2009). Bivalve aquaculture is one of the most sustainable methods to generate protein rich food and animal feed (Ackman 1989). Moreover, suspended mussel cultures can be used as a measure to remove nutrients from the marine environment through harvesting (Lindahl et al. 2005; Petersen et al. 2012, 2014). Since this type of production is optimized with respect to nutrient removal, the stock densities are high and the mussel sizes are relative small, and the low quality of the harvested mussels is mostly suited as feed for husbandry (Lindahl et al. 2005). This is in contrast to commercially suspended mussel cultures that aim to maximize the production of a uniform crop of large mussels with high quality for human consumption (Ferreira et al. 2007).

The harvested mussels must not be severely contaminated by pollutants posing a potential health risk for the consumers according to the European Union (EU) Directives for animal feed (Directive 2002/32/EC) and human consumption (Commission Regulation (EC) no. 1881/2006). The aquaculture producers exert political pressure to reduce contaminant inputs and maintain good water quality (Olsen et al. 2008; Duarte et al. 2009). Environmental Quality Standards (EQS) for metal concentrations in aquatic

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environments have been set for obtaining a "Good Ecological Status" (GES) in coastal waters according to the EU Water Framework Directive (WFD, Directive 2006/60/EC) and with regard to good chemical status in the revised priority substance daughter directive (Directive 2013/39/EC). In this paper, we use the GES term, which considers only the chemical status of metals. The applied EQS levels are above the unpolluted background level, but below the moderate status where there is risk of damage on the biota (Lepper 2005; SFT 2007). The metal level causing damage to marine organisms is probably higher than the appropriate level for a healthy marine mussel production, as the background assessment levels (OSPAR 2005) of especially Cd and Pb are a factor of 8–76 below the EQS in the WFD, despite the fact that the EQS was lowered from 7.6 (2008/105/EC) to 1.3 mg m^{-3} for Pb in 2013. The background value set by OSPAR is from areas influenced by diffuse, long-range transport of metals in dissolved form, whereas the value set by the Norwegian Environment Agency (SFT) is from total metal concentrations in areas with little or unsubstantial pollution. This problem with high differences between expected background and GES indicator values may also be valid for other marine waters with similar EQS, e.g. the Clean Water Action Plan in the US (Clean Water Act, Section 304 (a)) and the open waters within the EU according to the EU Marine Strategy Framework Directive (MSFD, Directive 2008/56/EC), given the need for the MSFD and WFD to be coherent. This suggests that the present GES levels in marine systems may need further testing in order to ensure that they agree with the requirements for mussel production.

Mussels exposed to trace metals in ambient seawater can accumulate high amounts of metals in their tissue (Wang et al. 1995). The metals are bioaccumulated in the mussels through dissolved uptake and particular ingestion and eliminated by depuration processes (Luoma and Rainbow 2005). The metal body burdens in blue mussels are long-term integrators of the environment and, as such, are often used as indicators for the status of metal pollution in coastal and estuarine waters (Phillips 1977). The metals cadmium (Cd) and lead (Pb) are considered as biologically non-essential and hazardous because they can exert various biological effects and threaten marine life (OSPAR 2010). The metals can be lethal at acute exposure, and long-term sub-lethal exposure can stress the organisms causing altered growth, filtration efficiency, oxygen consumption, enzyme activity and behaviour (Naimo 1995). Other metals, such as copper (Cu), nickel (Ni) and zinc (Zn), are essential nutrients for the organisms, but may become harmful above critical levels (Rainbow 2007). Metals are naturally occurring at background levels and can, in addition, be leaked into the marine systems from industrial and municipal wastewater discharges, atmospheric deposition, accidental spills, freshwater sources, precipitation and local runoff (Naimo 1995; Kim et al. 1999; Nobles and Zhang 2015).

The metals are often deposited and accumulated in the sediment over time (Borg and Jonsson 1996; Rubio et al. 2000; Atkinson et al. 2007). Contents of Cd and Pb in sediments from the North Sea show no temporal trend and have an unacceptable ecological status along the coastline (OSPAR 2010). Benthic filter-feeders are in close contact with contaminants in the water–sediment interface, and it has been shown that the bioaccumulation is higher here than for suspended cultures (Loaiza et al. 2015). However, remobilization of sediment metals due to physical disturbance, biogeochemical processes and bioturbation also influence the metal concentrations in the water column (Andres et al. 1998; Bonaglia et al. 2013; Superville et al. 2014).

Additionally, metal pollution can be influenced by climate change through higher temperatures, higher wind speeds, and higher precipitation and fresh-water inflow (Kim et al. 1999; Neumann 2010; Skogen et al. 2011). Temperature can affect metal uptake and depuration and physiological rates of mussels, which influence the body burdens of metals (Mubiana and Blust 2007; Sokolova and Lannig 2008). Wind induced physical disturbance causes re-suspension of particular-bound metals and higher release of dissolved metals from sediment pore water (Eggleton and Thomas 2004; Atkinson et al. 2007), and increased precipitation and fresh-water inflow may enhance the exposure to metals in the system (Kim et al. 1999). Hence, the effects of climate change should be taken into account when predicting the harvest quality of suspended mussel cultures.

Bioaccumulation modelling is commonly applied to quantitatively estimate the processes of metal accumulation and depuration and to predict metal burdens in shellfish from environmental concentrations (Wang et al. 1996; Luoma and Rainbow 2005). The modelling is often mechanistically based, but empirically considers differences in biology and between metals (Luoma and Rainbow 2005). Especially the assimilation efficiency of metals is a critical parameter for a correct estimation of the metal uptake by mussels (Fisher et al. 1996; Wang and Fisher 1999; Bourgeault et al. 2011). This type of modelling needs input on food uptake and growth, often obtained from simple calculations based on the observed size and biomass of the organisms on coarse time-scales. Another possibility is to use output from growth models with a higher temporal resolution. In this respect, Dynamic Energy Budget (DEB) models provide a detailed description of the physiological processes (filtration, food uptake, energy allocation, growth, and spawning) in response to the environment over time (Casas and Bacher 2006; Kooijman 2010).

The present study is part of larger study investigating the use of mussel mitigation cultures for nutrient extraction and as feed for husbandry in two full-scale farms located in a Danish estuary. The objectives of the present study were to i) estimate the bioaccumulation of cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) in suspended blue mussels (*Mytilus edulis* L.) based on observations and modelling, ii) compare the estimated body burdens of metals in suspended cultures with those of benthic mussels in the area and iii) assess the suitability of suspended blue mussels as animal feed in model scenarios using sea water metal concentrations corresponding to GES in the WFD and in climate change scenarios (higher metal concentrations and higher temperatures). The hypotheses were that 1) suspended mussels are less polluted than benthic mussels and 2) GES levels and climate change will increase the body burdens of metals in suspended mussels.

2. Materials and methods

2.1. Study site and sampling

Two commercial-scale mussel farms (M1 and M2) were rented for experimental purposes. The mussel farms were located in the Skive Fjord, the Limfjorden, Denmark (Fig. 1). The farms were 250 × 750 m large and water depths below the farms ranged from 6 to 9 m. Vertical mussel loops were attached to the long-lines at 0.4 m spacing and extended to a maximum depth of 3 m below surface water. The Limfjorden is a shallow, semi-enclosed estuary located between the North Sea and the Kattegat (Baltic Sea) with low tidal amplitude. Salinity varies from 32 to 34 in the Western part to 19–25 in the Eastern part. The fjord is eutrophic, receiving nutrients from the catchment area which is dominated by agriculture (Maar et al., 2010; Tomczak et al., 2013). The system supports a high stock of blue mussels, *M. edulis* L., and a thriving mussel fishery varying from 40,000 to 50,000 t per year. Farming of long-line blue mussels is being tested in the fjord, and in recent years this production has been in the range of 500–630 t per year.

Sampling of temperature, salinity, Chlorophyll *a* (Chl *a*), and

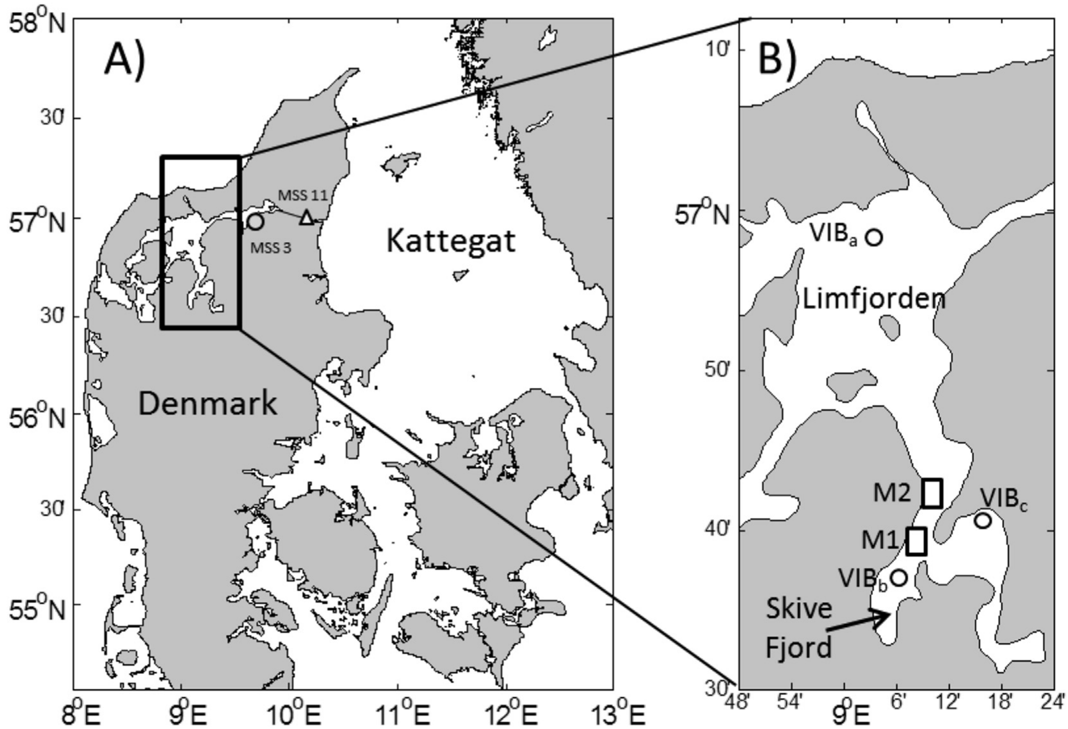

Fig. 1. Maps showing A) location of the Limfjorden in Denmark with the position of the two monitoring stations MSS 11 (polluted) and MSS 3 (reference) of benthic mussels and B) position of the two mussel farms (sites M1 and M2) and the three monitoring stations (VIB_a, VIB_b and VIB_c) of benthic mussels.

metals in water and blue mussels was conducted at two sites during different periods; from July, 2010, to May,2011, at site M1 and from August, 2012, to June, 2013, at site M2. The collected field data was used as input to the model of the two sites. Vertical profiles of temperature and salinity were measured within the farms (22 times at M1 and 12 times at M2) using a calibrated CTD (ECO-probe; Meerestechnik, Elektronik GmbH, Trappenkamp, Germany). Water samples for Chl *a* determination were collected within the farms (29 times at M1 and 12 times at M2) with a 1.7-L Niskin sampler from a depth of 1 m below surface. During periods with ice cover, sampling was less frequent. Water samples were kept in the dark during transport to the laboratory, where they were immediately pre-filtered through a 200 μm mesh to avoid inclusion of mussel faecal pellets. The pre-filtered water samples were then analysed for Chl *a* by filtering either 50 ml triplicate sub-samples through Whatman glass fiber filters (0.7 μm nominal pore size). The filters were extracted in 10 ml of 96% ethanol and analysed using a Turner fluorometer that was calibrated against a Chl *a* standard. We used a C:Chl *a*-ratio = 30 for conversion to carbon food (Riemann et al. 1989).

Mussel samples were collected by removing approximately 1-m sections of mussel loops at predetermined sampling locations within the farm. On each sampling occasion (7 times at M1 and 10 times at M2), 3 × 1 m subsamples from both bottom and top of the mussel rope were collected. For each subsample, shell length was measured on 100 randomly selected mussels with a digital caliper (±1 mm) and individual tissue dry-weight (DW; ±1 mg) was measured on 30 (M1) and 50 (M2) randomly selected mussels. Mussel biomass (all soft parts) and shell were separated, and each were dried at 80 °C for ≥4 d. DW-samples were cooled to room temperature in a desiccator before weighing to the nearest mg. For gonad analysis, 50 mussels were selected randomly. If either sperm or eggs were detected in the gonads, they were separated from the mantle cavity, and dried separately at 80 °C for ≥3 d.

In order to compare the metal concentrations in the suspended mussels with benthic mussels from nearby areas in the Limfjorden, we compiled data from the Danish National monitoring program NOVANA (Larsen and Strand 2014). The two stations with the longest time-series in the Limfjorden, Nibe Bredning (MSS 3) and Langerak (MSS 11), were selected (Fig. 1) and used as examples of the temporal development of metal concentrations in mussels from 1999 to 2013. MSS 3 represent an unpolluted reference station with no major cities or industrial areas close by. MSS 11 is located approx. 20 km east of Denmark's 4th largest city, Aalborg (106,000 inhabitants), and 8 km from the Kattegat entrance to Langerak. It is close to the shipping route through Langerak and, hence, expected to be more affected by anthopogenic input than MSS 3. In addition, samples (2007–2014) from the monitoring stations VIB_a ($n = 6$), VIB_b ($n = 5$) and VIB_c ($n = 2$) were pooled (VIB) and considered as representative for benthic mussels in the farm area. All samples were analysed by the same method as described for the suspended mussels. Trends in time-series were tested using correlation analysis ($\alpha = 0.05$). Difference between means of metals in suspended versus benthic cultures were tested using *t*-tests ($\alpha = 0.05$). Both tests were performed using Microsoft Excel version 14.2.0.

2.2. Metal analysis

Water sampling for metals (dissolved and particular phases) was performed using a sampler made of Teflon, sampling directly into 550 ml Teflon bottles, by releasing two Teflon plugs in 10 cm silicone tubing using a nylon rope at the desired depth of sampling (1 m). The samples were kept at room temperature and acidified with 1 ml of Suprapur HNO_3. Salt interference was removed by diluting samples with buffer solution of acetic acid to pH 4.2 and collecting metal ions on a chelex-100 resin, following stripping using a 10% HNO_3 solution and recording of transient signal from the chelex-100 resin using an ICP-MS (Agilent 7500ce) according to

Søndergaard et al. (2015). While loading the resin, carrier gas was set to 0, effectively draining away the sample with high salinity, and turning back on the carrier gas before stripping of metals from the resin. Quantification was done by addition-calibration using certified reference materials NASS-4 and validated by CASS-4 and SLEW-3 certified reference materials (All from National Research Council of Canada (NRC – CNRC)). The Danish Accreditation board DANAK accredits this method. The precision is better than 5% at 10 mg m^{-3} spike and recovery 93–101%. Detection limits range from 5 (Cd) to 159 ng/l (Zn) for the elements reported, and the method is evaluated twice per year by participation in an international proficiency testing scheme (Søndergaard et al. 2015).

Metals in the water phase were also measured using diffusive gradients in thin films (DGT) passive samplers (Zhang and Davison 1995; Montero 2012). The samplers were deployed for 6 weeks at four positions in the mussel farm at 1 m depth, coinciding with spot sample collection. Ready-made DGTs from DGT Researchs (Lancaster; www.dgtresearch.com) with 0.8 mm diffusion layer thickness were purchased and deployed as received. After collection, the chelex-gel receiving phase was extracted with 2 ml half concentrated suprapure Nitric Acid for 24 h and marked up to 10 ml with 18 MOhm millipore water. The final extract was analysed using the same method as for mussels below. The measured extract was recalculated to labile water concentrations C_{DGT} according to (Zhang 2003), taking into account average temperatures measured with thermo-loggers deployed simultaneously. The C_{DGT} concentration is expected to be representative of the dissolved (bioavailable) concentration of metals (Montero 2012).

Subsamples of mussels for metal analysis were taken together with samples for growth estimates as described above. Pools of approx. 20 mussels were dissected and frozen immediately, and the soft parts were freeze-dried. The dried samples were homogenized by hand in a mortar or ball-mill. Individual mussels were dissected and the shells, gonads and other soft-parts were freeze-dried and analyzed separately. To approx. 0.4 g of each sample was added 20 ml of half concentrated Suprapur HNO_3, which was digested using closed vessel microwave oven (Multiwave, Anton-Paar) as previously described (Larsen et al. 2011) and analysed using ICP-MS (Agilent 7500ce) with external standardisation, drift correction using In, Ir, Rh, Ge internal standards and an octopole reaction chamber for minimizing interferences using He and H_2 gas phase reactions with the ion beam.

2.3. Dynamic Energy Budget model

The DEB model (Fig. A1) was used to describe clearance rate, ingestion and growth of blue mussels in response to temperature and food that was used as input to the bioaccumulation model for metals (see next section). The DEB model was, with some modifications (Table A1), parameterised according to previous studies (van der Veer et al. 2006; Saraiva et al. 2011). In the DEB model, mussels filter water for food with a clearance rate (*CR*, m^3 d^{-1}), depending on mussel size, food concentration, C_f (mg m^{-3}), and a temperature correction function (T_{corr}, Fig. 2A):

$$CR = CR_m \times V^{2/3} \times \frac{C_f}{\left(C_f + X_k\right)} \times T_{corr} \tag{1}$$

where CR_m is the maximum *CR*, *V* (cm^3) is the structural volume of the mussel, and X_k is the half-saturation coefficient (mg m^{-3}) (Table A1). The *CR* fitted best to experimental data from mussels exposed to algae monocultures (Riisgård et al. 2013) using $X_k = 1$ mg-Chl *a* m^{-3} (Fig. 2B). However, we used $X_k = 3$ mg-Chl *a* m^{-3} (Fig. 2B) in the model because this gave a better fit to field data.

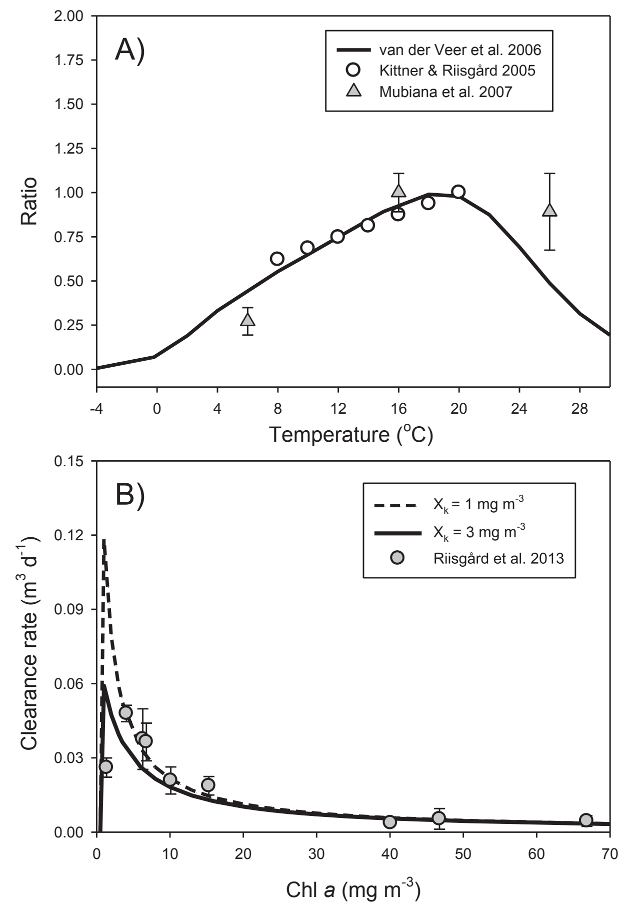

Fig. 2. Parameterizations of temperature and *CR* responses in the DEB model. A) The model temperature correction factor from 0 to 1 (van der Veer et al. 2006) shown together with experimental values for *CR* at the temperature interval from 8 to 20 °C (Kittner and Riisgård 2005) and Cu-depuration at three different temperatures (Mubiana and Blust 2007) both normalized by their maximum value. The temperature correction is expressed as $T_{corr} = \exp\left(\frac{T_A}{T_{opt}} - \frac{T_A}{T}\right) \times \left(1 + \exp\left(\frac{T_{AL}}{T} - \frac{T_{AL}}{T_L}\right) + \exp\left(\frac{T_{AH}}{T_H} - \frac{T_{AH}}{T}\right)\right)^{-1}$ where *T* is the temperature in Kelvin, see Table A1 for parameters. B) Clearance rate as a function of Chl *a* concentrations for a 3.0 cm mussel at two different X_K and the measured *CR* by Riisgård et al. 2013.

This difference was probably due to a more complex diet composition in the field or re-filtration of the water by mussels within the farm (Pouvreau et al. 2006; Maar et al. 2008). The ingestion rate (J_x, mg d^{-1}) is described as:

$$J_x = CR \times C_f \tag{2}$$

Observed growth ceased or was negative during the ice cover periods, probably because the mussels closed their valves at low temperatures (Kittner and Riisgard 2005) or because ice cover limited physical transport of food to the mussels. In order to describe this reduced growth, we adjusted the ingestion rate to 10% of maximum during the ice cover periods at both sites. The ingested food is assimilated by constant assimilation efficiency (AE_f) in the model. The assimilated food goes to the reserve density (*E*, mol-C cm^{-3}) and from there energy is allocated with the fraction *K* to somatic growth (structural volume) and maintenance and the fraction (1- *K*) to maturity or reproduction (*R*, mol-C) and maintenance (van der Meer 2006; Kooijman 2010). Spawning takes place above a temperature threshold and above the somatic-gonado index (GSI = gonads/total SP). The reserve density-ratio

(E/E_m) reflected the nutritional status of the mussels (from 0 = severe starvation to 1 = no food limitation). Gonad data showed that long-line mussels reached a longer shell length at the age of maturity than bottom populations due to faster growth. Consequently, we increased the structural volume of sexual maturity from 0.06 cm^3 (1.2 cm) to 0.65 cm^3 (2.8 cm) in the DEB model. Biomass, B (g-DW), of the mussels is estimated as:

$$B = V \times DWV + E \times V \times DWC + R \times DWC \quad (3)$$

The first term on the right side is the biomass of somatic tissue, where structural volume (cm^3) is converted to biomass (g-DW) using the factor DWV (Table A1). The second term is the biomass of reserves and the third term is the biomass of reproductive tissue, where DWC is the conversion factor from mol-C to g-DW (Table A1). Shell length, SL (cm), is estimated from:

$$SL = V^{1/3} \times \delta_v^{-1}, \quad (4)$$

where δ_v is the shape factor (Table A1). The DEB model was forced by linear interpolated measurements of temperature and Chl a. The DEB model results of shell lengths, biomass and gonads were compared with observations from the two study sites using correlation analysis ($\alpha = 0.05$).

2.4. Bioaccumulation model

Metals are available to mussels through both dissolved and particulate phases. In the present study, we measured total metal concentrations of both phases in the same water samples and the applied assimilation efficiency; the resulting AE_m (ratio) was an average of both phases. Changes in the metal content of mussels, M_{mussel} (mg kg^{-1}) per time step, dt (d^{-1}), were described by the bioaccumulation model:

$$\frac{M_{mussel}}{dt} = \frac{CR \times C_m \times AE_m}{\left(\frac{B}{1000}\right)} - (K_e + g + u_{shell} + spawn) \times M_{mussel} \quad (5)$$

The first term on the right site of Equation (5) describes the metal uptake that was assumed to be proportional to the CR (m^3 d^{-1}), sea water metal concentrations, C_m (mg m^{-3}), and the AE_m divided by the mussel biomass. The losses of metals are described in the second term of Equation (5). The metal content in the mussels decreases due to depuration, K_e (d^{-1}), when the mussels grow, g (d^{-1}), by uptake in the shell, u_{shell} (d^{-1}), and for some metals during spawning, $spawn$ (d^{-1}). CR, B, g, and spawning were derived from the DEB model for each time step. The amount of metals lost during spawning was obtained from the measured metal content in gonads. The depuration rate K_e for each metal was obtained from literature values and varied from 0.014 to 0.022 d^{-1} (Table 1). For Cu, temperature correction (Fig. 2A) was applied on the depuration rate according to a previous study (Mubiana and Blust 2007). During ice cover, the depuration rate was set to 10% of the maximum for all metals to account for the reduced activity of the mussels. The AE_m is known to be site-specific (Bourgeault et al. 2011). It was therefore necessary to estimate the AE_m values through model calibration by tuning the model against observations (Fig. 4, Table 1). The metal uptake by shells was assumed to be proportional to the increase in structural volume (dV/dt) derived from the DEB model. The biomass of shells, B_{shell} (g-DW), was estimated from the empirical relationship (n = 57, R^2 = 0.91, $p < 0.05$):

$$B_{shell} = 0.1219 \times \exp^{(0.595 \times SL)} \quad (6)$$

2.5. Model scenarios

In the GES model scenario, we tested if the EQS targets for GES in the environment (Table 2) also corresponded to the targets set for the biota. We used the EQS targets for total metal concentrations (dissolved and particular) from the SFT that is consistent with our study, whereas WFD targets are for the dissolved phase (Table 2). The SFT target for Ni is more protective than WFD, similar for Cd and Pb and no WFD targets have been set for Cu and Zn. In the climate change scenarios, we used an increase of 3 °C in sea water temperature, according to the A1B green gas emission scenario (IPCC 2007) applied to the Baltic Sea (Neumann 2010). Furthermore, higher wind speeds (up to 50%) and higher precipitation and fresh-water inflow (on average 20%) were predicted in the A1B scenario for the Baltic Sea (Neumann 2010). In the climate change scenarios, we assumed that this would cause an overall increase in sea water metal concentrations of 50% due to higher inflow of metals with fresh-water and remobilisation of sediment metals (Kim et al. 1999; Eggleton and Thomas 2004; Atkinson et al. 2007). In summary, model scenarios were conducted to evaluate the metal content of suspended mussels exposed to:

i) metal concentrations corresponding to GES in the WFD
ii) at 50% higher environmental concentrations
iii) at 3 °C higher temperatures
iv) the combination of 3 °C higher temperatures and 50% higher concentrations.

The measured and model estimated body burdens of Cd and Pb were assessed against the safety limits in the EU Directives for animal feed (Directive 2002/32/EC) and human consumption (EC no. 1881/2006). The given thresholds were converted from WW to DW using a water content of 12% in the animal feed (Directive 2002/32/EC) and 20% in human food products (present study). In addition, the contents of Pb, Cd, Cu and Zn in mussels were assessed against the OSPAR "Background Assessment Concentrations"

Table 1
Model parameters for the different metals of AE_m for the two sites and literature (lit.) values on absorption efficiency for dissolved phase (AE_d), assimilation efficiency for particulate phase (AE_p) and K_e for different bivalves. The K_e values were applied in the bioaccumulation model with temperature correction for Cu.

Metal	Model M1 AE_m (%)	Model M2 AE_m (%)	Lit. AE_d (%)	Lit. AE_p (%)	Lit. & model K_e (d^{-1})	Species	References
Cd	4.5	6.0	0.31	11–40	0.014	*M. edulis*	Wang et al. 1996
Cu	1.0	1.4	–	2–26	0.022	*Dreissena polymorpha*	Mersch et al. 1993, Bourgeault et al. 2011
Ni	0.6	0.1	–	3–14	0.019	*M. edulis*	Zaroogian and Johnson 1984, Bourgeault et al. 2011
Pb	1.6	0.5	–	56–64	0.020	*M. gallo-provincialis*	Fisher et al. 1996
Zn	3.2	7.0	0.89	16–48	0.015	*M. edulis*	Wang et al. 1996

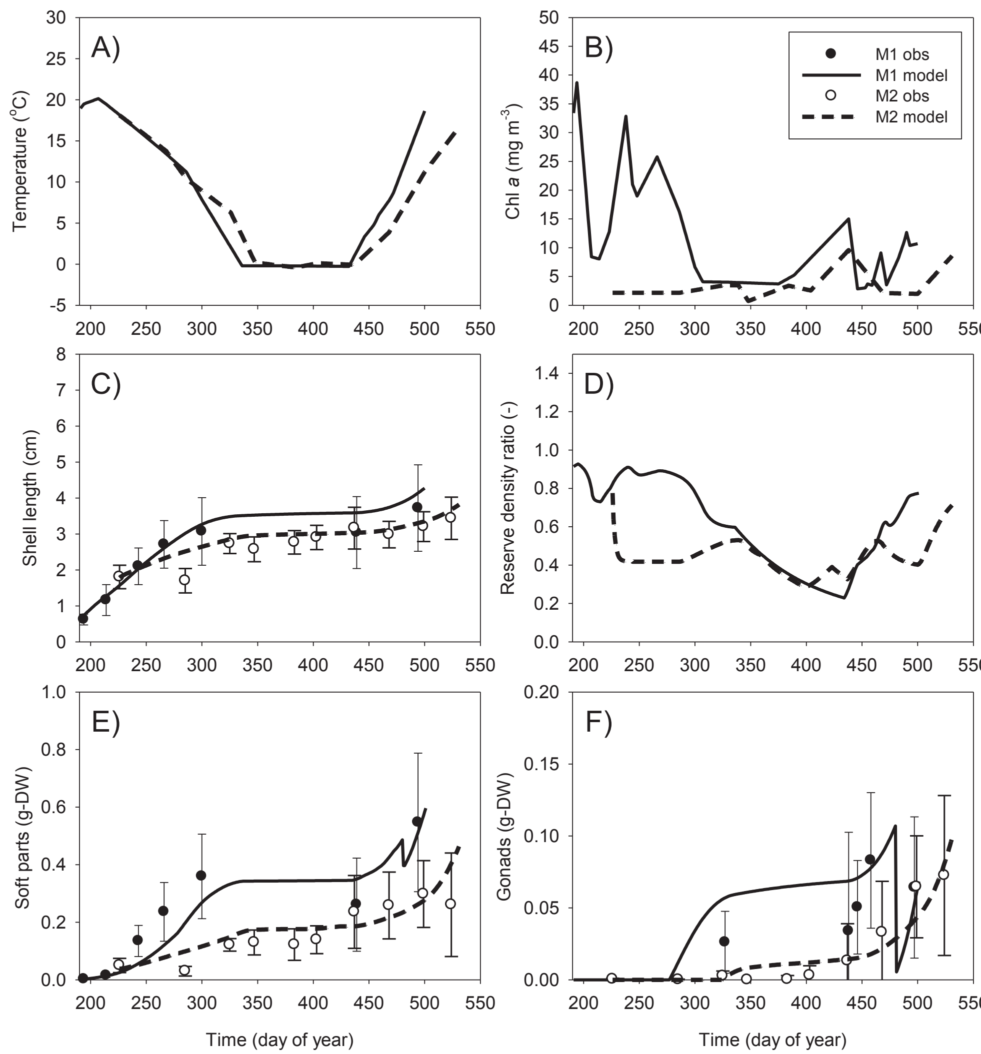

Fig. 3. Interpolated measurements of temperature (A) and chl *a* (B) used as model forcing. Observed average ±SD (spheres) and model results (line) of shell length (C), reserve density ratio (E/E_{max}) (D), total biomass (E) and gonads (F) of mussels from July 2010 to May 2011 at site M1 and from August 2012 to June 2013 at site M2.

(BAC's) (OSPAR 2005, 2014). Background concentrations are intended to represent the concentrations of pollutants that would be expected in the North-East Atlantic if certain industrial developments had not happened (OSPAR 2005). The BAC is the background concentration adjusted for the standard analytical uncertainty to produce a limit that can be used for statistical tests. Ni was not included in the OSPAR assessment, and we used the "insignificant to slightly pollution" concentration defined by SFT (1997) as a proxy for the OSPAR BAC. The SFT BAC concentrations are 2–4 times higher than the OSPAR BAC for Cd, Cu, Pb and Zn, because SFT includes concentrations "far away from known point sources" in Norway. The Norwegian classification system uses 4 classes (apart from BAC) ranging from "moderate pollution" to "very strong pollution", with moderate pollution as the border for acceptable status (SFT 1997; SFT 2007).

3. Results

3.1. Environmental data

The Skive Fjord was covered by ice from 2nd December, 2010, to 15th March, 2011, (site M1) and from 20th January, 2013, to 13th March, 2013, (site M2). Temperatures varied from −0.2 °C during the ice cover period to 20 °C during summer for both sites (Fig. 3A). Salinity varied from 17 to 25 (data not shown). Average (±SD) Chl *a* concentrations were significantly higher (*t*-test, $df = 40$, type 2, $p < 0.01$) at site M1 (13.6 ± 10.2 mg m^{-3}) than at site M2 (3.5 ± 2.6 mg m^{-3}) (Fig. 3B).

Seawater concentrations of Cd and Zn were significantly higher and Ni significantly lower at site M1 in comparison with M2, whereas there was no significant difference between Cu and Pb concentrations (Table 2). Cd and Cu seawater concentrations were below the natural background levels at M2 (Table 2). Cd, Ni, Pb and Zn concentrations were below the threshold of GES according to the WFD and SFT (Zn). At site M1, Cu concentrations were above the threshold for moderate status according to SFT, indicating a marked pollution from e.g. local point sources and, potentially acute toxic response.

DGT samplers were deployed to measure the dissolved metal concentrations in the water in the spring period at M1. Comparisons between the total water samples and DGT average (±SD) concentrations indicate that dissolved Ni and Cd concentrations was 52 ± 18% of the total, Pb 18 ± 17%, Zn 12 ± 6% and Cu 9 ± 3% of the total water concentrations samples from April to mid-May. The relative SD of the 4 spot samples was 4–11% for Ni, 34–44% for Cd, 50–71% for Cu and Zn and 55–134% for Pb, indicating that

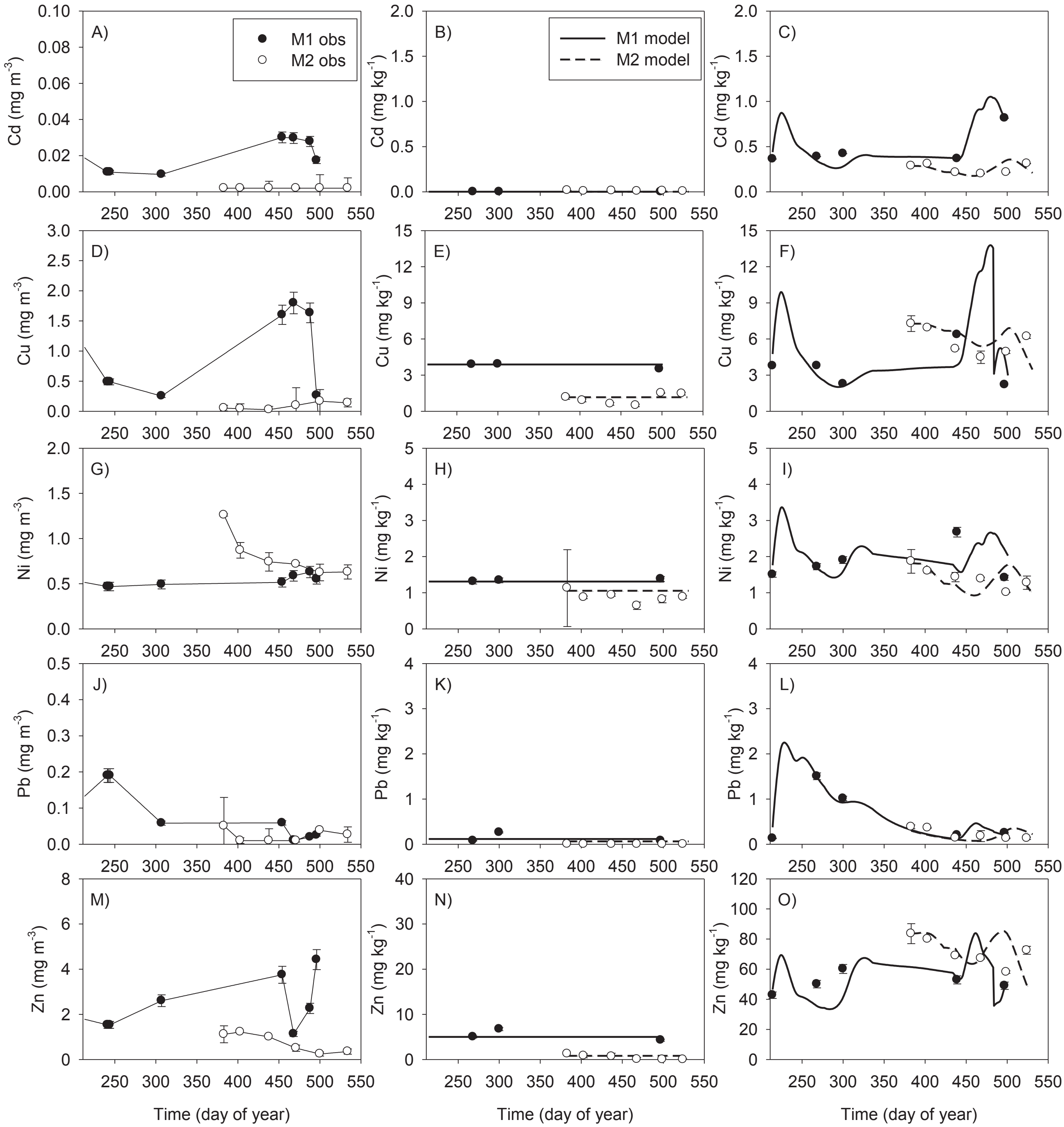

Fig. 4. Measured sea water concentrations linearly interpolated (1st column), measured (spheres) and modelled (line) metal content in shell (2nd column) and biomass (3rd column) for the metals Cd (A–C), Cu (D–F), Ni (G–I), Pb (J–L) and Zn (M–O) at sites M1 and M2.

particularly Cu, Pb and Zn varied widely within the 42 days of deployment.

3.2. Growth of suspended mussels

Observations showed that blue mussels obtained a higher shell length (3.72 ± 1.2 cm) and biomass (0.55 ± 0.24 g-DW) in May, 2011, at site M1 than in June, 2013, at site M2 (3.44 ± 0.59 cm and 0.26 ± 0.18 g-DW) (Fig. 3C, E). The model predicted spawning to occur early May at site M1, but not before harvest time at site M2 (Fig. 3F). The modelled reserve density-ratio (0.61 ± 0.23) showed that mussels were in a relatively good condition at site M1, except during the period with ice cover (Fig. 3D). This ratio was generally lower (0.44 ± 0.09) at site M2, indicating food limitation (Fig. 3D). There were significant correlations between observations and model results of shell length, biomass and gonads (Table A2).

Table 2
Seawater dissolved concentrations (mg m^{-3}) of metals that correspond to Background (OSPAR 2005; Southern North Sea) and good chemical status (AA-EQS 2013/39/EC) and "Background", Good and Moderate status for SFT 2007 (total metal concentrations). The average ±SD total metal concentrations from the two study sites are shown in the last 2 columns. Values in bold are above the criteria for AA-EQS or SFT Good (Cu, Zn). A significant difference in means of metals is shown as '*' between the two sites (*t*-tests, type 2, $df = 11$, $p < 0.05$).

Metal	OSPAR/SFT background	AA-EQS/SFT# good	SFT Moderate	This study M1	This study M2
Cd	0.012/0.03	0.20/0.24	1.50	0.02 ± 0.01	0.002 ± 0.001*
Cu	0.36/0.30	–/0.64	0.80	**0.88 ± 0.67**	0.10 ± 0.07
Ni	0.26/0.50	8.60/2.2	12.00	0.52 ± 0.06	0.81 ± 0.24*
Pb	0.017/0.05	1.30/2.2	2.90	0.09 ± 0.08	0.02 ± 0.02
Zn	0.28/1.50	–/2.90	6.00	2.35 ± 1.18	0.77 ± 0.44*

3.3. Metal content in suspended cultures

In the gonads, there was accumulation of Cu, Pb and Zn of 77%, 7% and 41% relative to the biomass metal content, respectively, measured in May, 2011 (Table 3). There was no accumulation of Cd and Ni in the gonads. For shells, the measured metal content of Cu, Ni, Pb and Zn varied from 9 to 58% of the total (biomass + shell) metal content (Table 3). The Cd concentration in the shells was below detection limit, hence, set to 0. The temporal development in metal content of the shells (mg/kg) was rather steady according to measurements at both study sites (Fig. 4). At site M1, metal content in mussel biomass was more variable over time than at site M2 (Fig. 4), but was within the same order of magnitude (Fig. 5). The average deviation between measurements and modelled biomass metal content was 36% and 35% at site M1 and M2, respectively. All model estimates of metals in biomass were within ±2 × deviation from the 1:1-ratio (Fig. 5). The AE_m (dissolved and particulate phases) was obtained through model calibration and varied from 0.1% to 7.0% (Table 1).

The measured metal contents in mussel biomass of Cd and Pb were below the critical threshold for both animal feed (Directive 2002/32/EC) and human consumption (EC no. 1881/2006) at harvest time at both study sites (Fig. 6). The metal contents in mussels were all below the BAC's and within the moderate polluted class defined by SFT (SFT 1997).

3.4. Metal content in benthic mussels

There were no significant time trends for the metal contents at MSS 3 and MSS 11, except for Cd at MSS 11 showing a significant increasing time trend of ~0.05 mg kg^{-1} $year^{-1}$ from 2003 to 2013 (Fig. 7). The coefficient of variation (CV=SD/mean) varied from 16 to 45%. The concentration levels of all metals in benthic mussels were generally higher than for suspended farm mussels in agreement with hypothesis 1 (Fig. 6). At the station MSS 11, all the metal contents were significantly higher than in the suspended mussels (*t*-test, type 2, $p < 0.001$). The reference station MSS 3 and the VIB station (representative for the farm area) showed higher metal contents of Cd, Cu and Zn in comparison with suspended mussels (*t*-test, type 2, $p < 0.02$). The Cu, Pb (only MSS 11) and Zn content in benthic mussels were above the BAC's, whereas all metals were below the upper SFT class for moderate pollution (Norwegian GES) (Fig. 6).

Table 3
Measured average (±SD) content of metals in gonads (% of SP) in May, 2011, at site M1 and shell (% of shell + SP) as an average of three sampling occasions at site M1.

Metal	% Gonads	% Shell
Cd	0	0
Cu	77 ± 40	58 ± 7
Ni	0	45 ± 4
Pb	7 ± 6	12 ± 8
Zn	41 ± 29	9 ± 1

3.5. Model scenarios

The results of the model scenarios and the comparison with safety limits for body burdens of metals are shown in Fig. 6. In general, the GES and climate change scenarios caused the body burdens to increase in agreement with hypothesis 2. The GES scenario showed the strongest response, and the content of all metals exceeded the BAC's. Furthermore, the metal content of Cd and Pb exceeded the safety limits for animal feed and human consumption, and Cd, Ni and Pb exceeded the SFT moderate pollution level. A 50% increase in metal concentrations increased the body burdens (45–66%) for all metals, and Cu and Zn exceeded the BAC's. Higher temperatures (+3 °C) increased the body burdens by 18%–37% for Cd, Cu, Pb and Zn, but only the change in Zn concentrations exceeded the BAC. The change was negative for Ni (−6%) due to fast growth just before harvest time, which was not followed by the

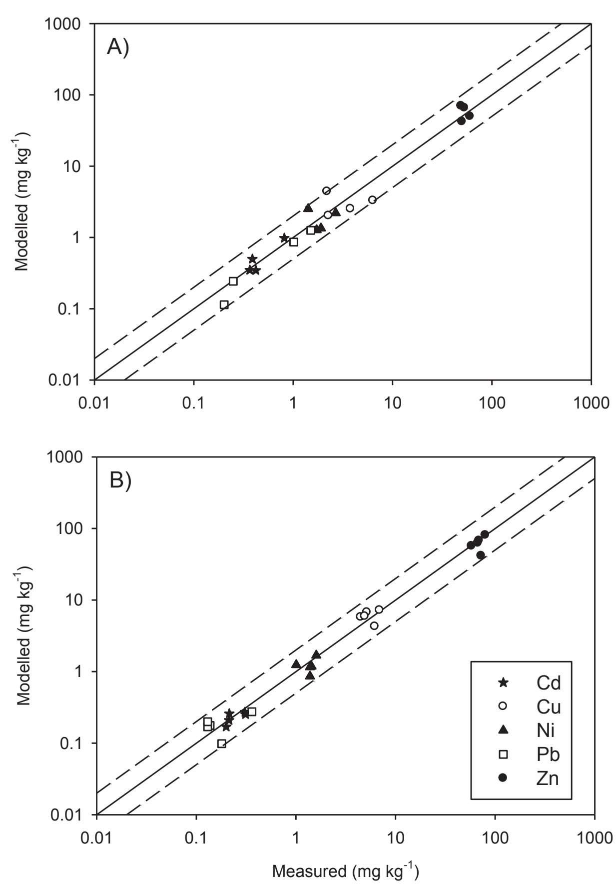

Fig. 5. Comparison of measured and modelled metal content in mussels for A) mussel site M1 and B) mussel site M2. The solid line is the 1:1-ratio and the dashed lines are the ±2 × deviation from the 1:1-ratio.

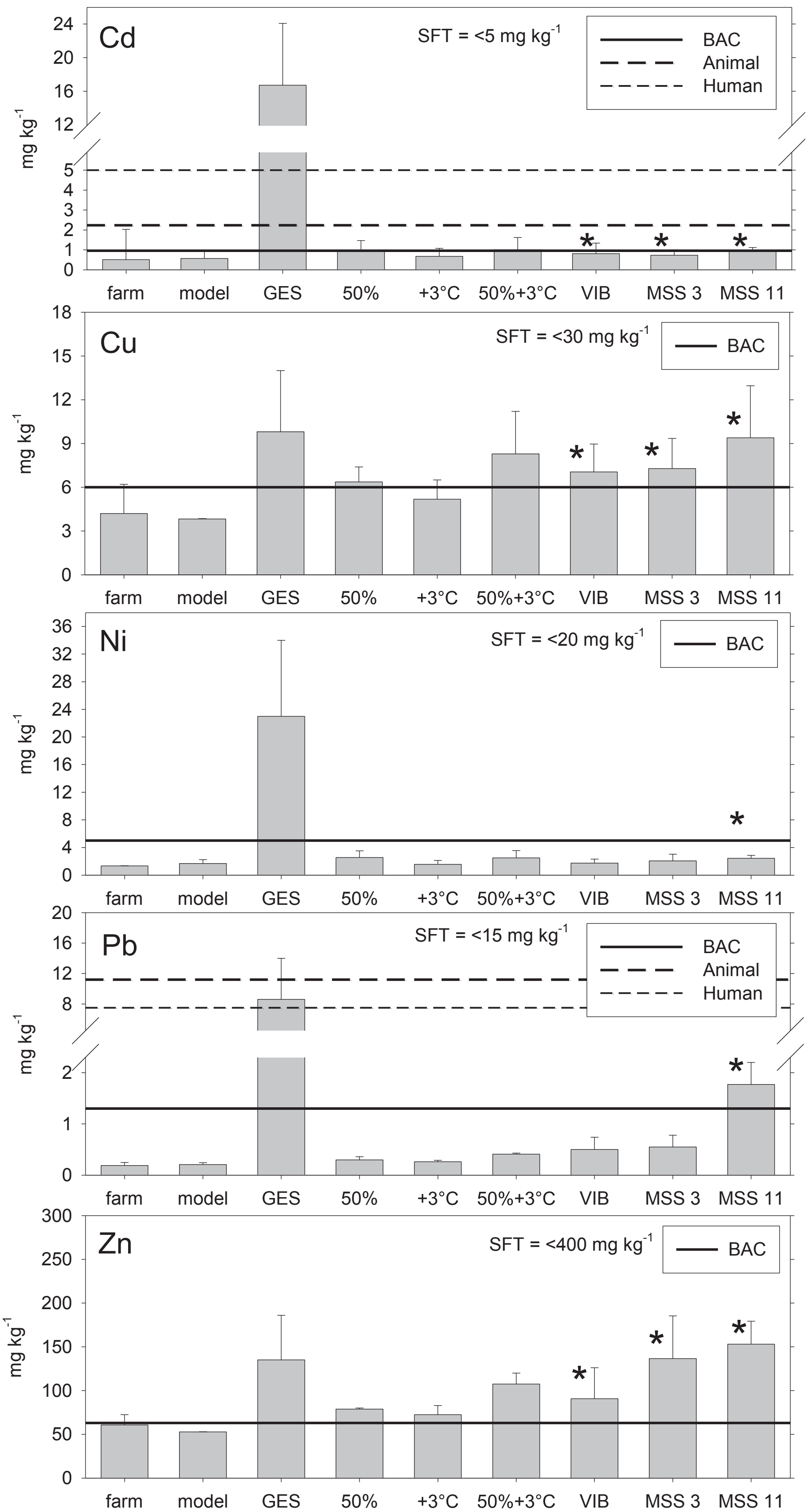

Fig. 6. Average (±SD) metal content (mg kg^{-1}) in suspended mussels from observations and model data from the two farm sites. Results of model scenarios of mussels exposed to concentrations according to GES, 50% higher metal concentrations, 3 °C higher temperatures, and a combination of +3 °C and 50% higher concentrations. The last three columns are the average (±SD) metal content in benthic mussels at the monitoring stations VIB (representative for the area), MSS 3 (reference) and MSS 11, where '*' is a significant difference from the suspended mussels (*t*-test, type 2, $p < 0.05$). Safety limits for metals according to EU directives for human consumption (human) and animal feed (animal) for Cd and Pb and BAC's for all metals are shown as horizontal lines. The thresholds for moderate pollution defined by SFT (1997) are shown in text boxes.

same amount of Ni-uptake. The combination of higher background concentrations and higher temperatures increased the body burdens by 50–116% for all metals and thereby exceeded the BAC's threshold for Cu and Zn. The climate change scenarios (50% increase and +3 °C) did not cause the modelled concentrations in mussel tissue to exceed critical SFT thresholds or EC maximum limits.

4. Discussion

4.1. Good environmental status?

Metals are persistent pollutants in aquatic environments, especially in coastal areas exposed to a high degree of urban pressure (Rainbow 2007). The goal of the EU WFD (Directive 2000/60/EC) is to achieve GES in coastal waters defined by the EQS for pollutants posing a potential risk to the environment. In open waters, the targets for the chemical status are defined in descriptor 8 in the EU MSFD (2010/477/EU), and these are expected to be coherent with the WFD on regional scales due to the overlapping of water masses. The applied EQS targets for metals in aquatic systems have mainly been evaluated with respect to damage to the organisms (Lepper 2005; SFT 2007) and not in relation to the safety limits for metal content in harvested mussels used for human consumption and animal feed production.

The GES targets for seawater concentrations of Cd, Ni and Pb are 8–76 times higher than the BAC's and the 13 to 24 times higher than ambient concentrations in the Limfjorden (Table 2). In the GES model scenario, the higher ambient concentrations of Cd, Ni and Pb caused the bioaccumulation to exceed the critical levels for feed for husbandry and human consumption and the SFT moderate pollution class (Fig. 6). This suggests that the water EQS targets for GES of Cd, Ni and Pb in the WFD are not protective with respect to marine mussel production, and based on our model predictions, they should be reduced for marine waters (“other surface waters”) to protect mussel consumers. For Cu and Zn, the GES targets are closer to the BAC's (factor 2.0) and actual concentrations in the Limfjorden (factor 1.3–1.9). In the GES model scenario, the body burdens of Cu and Zn increased to the same level as for benthic mussels, but were, nevertheless, within the SFT class of moderate pollution, i.e. potentially impacted by local pollution, and some effects of the metals cannot be excluded (SFT, 1997). The revision in (SFT, 2007) only covers sediment and water, but here the moderate class includes concentrations that can cause chronic effects after long-term exposures. Altogether, this suggests that GES targets for metals that are much higher than the BAC's (>8 times) should be revised in order to secure a healthy mussel production. This finding is supported by another study of water metal concentrations in the Mediterranean (Paraskevopoulou et al. 2014). In this, it was found that due to natural attenuation, the high EQS values were not useful to classify the chemical status based on rough categories such as “good” or “not good”, as more sensitive species may still be affected and background values were far below EQS values (Paraskevopoulou et al. 2014). This may also apply to other coastal areas, for example the US Clean Water Plan for marine waters in North American (EPA 2015) has similar targets for Ni and even higher targets for Cd, Cu, Pb and Zn than GES in the EU WFD. The implication for these higher values is that areas found suitable for aquacultures based on water quality in reality may be unsafe in relation to human food and animal feed.

The ambient metal concentrations were generally below or slightly higher than the BAC's and far below the GES levels, except for Cu (Table 2). The measured body burdens (Cd, Cu, Ni, Pb and Zn) of mussels from suspended cultures were all below the BAC's and within the moderate (acceptable) pollution class by SFT (Fig. 6). Body burdens of Cd and Pb were, furthermore, below the safety limits according to the EU directives for animal feed and human consumption. Benthic mussels were more polluted by the metals Cd, Cu and Zn at all monitoring stations and also by Ni and Pb at the most polluted station in comparison with suspended mussels (Fig. 6). In most aquatic environments, dissolved metal concentrations in overlying waters are low due to precipitation as solids or adsorption to suspended particles and the deposition of these particles as sediments (Atkinson et al. 2007). This supports the theory that one of the advantages of suspended cultures is the lower contact with hazardous substances in the water–sediment interface and from re-suspended materials. However, short-term or repeated pulse events of metals in the water column, e.g. during rain events or industrial spills, have also been shown to have an effect on marine assemblages (Johnston and Keough 2000). Metal contents in benthic mussels were, nevertheless, below the safety limits (Fig. 6) and showed no temporal trends, except for the increase of Cd at the most polluted station (Fig. 7) probably due to influence by anthropogenic pollution from the nearby city. The content and temporal development of metals in benthic mussels can be used to identify areas that are constantly low in pollution level and, hence, can be used to expand the production of suspended mussels.

4.2. Climate change scenarios

When planning suitable locations for mussel mitigation cultures, it is important that the area is not polluted or constitutes a potential risk for future contamination of the mussels due to climate change. In the climate change model scenarios, wind-mixing was assumed to cause remobilisation of metals in the sediment due to higher re-suspension of particles and release of dissolved metals from pore water to the water column (Eggleton and Thomas 2004; Atkinson et al. 2007). In addition, increased precipitation and fresh-water inflow may cause higher ambient metal concentrations. A 50% increase in sea water metal concentrations gave a similar response (45–67%) in the bioaccumulation of metals in the model (Fig. 6). The 50% metal increase was assumed to be a realistic estimate, since it was close to the upper end of the natural year-to-year variability (16–45%) in ambient metal concentrations at the monitoring stations and since the climatic drivers changed 20–50%. The assumed increase in sea water metals is, nevertheless, very uncertain.

The metal concentrations were increased by 50% during the whole period, but this approach does not take the seasonal component or episodic events, e.g. storms, local spills or strong rain, into account. Short-term pulses of metals can e.g. influence settling and densities of marine invertebrates considerably (Johnston and Keough 2000, 2002). Metals from precipitation and run-off usually have the initial effect on organisms in the water column, with filter-feeders being subject to this exposure before any settlement of metals into the sediment (Johnston and Keough 2000). Furthermore, a decrease in salinity could change the bioavailability, metal uptake and growth of mussels (Luoma and Rainbow, 2005; Maar et al., 2015). In the model, the assimilation efficiency of metals was applied as an average estimate of dissolved and particulate phases without a seasonal distribution. In fact, the temporal patterns of ambient metal concentrations and bioavailability could influence the predictions of biological responses to climate change (Montalto et al. 2014).

The relation between physical forcing and metal release from the sediment and pore water is largely unknown (Eggleton and Thomas 2004; Atkinson et al. 2007). The metal release from sediments depends on many factors such as the metal content and residence time in the sediment, metal bioavailability, sediment

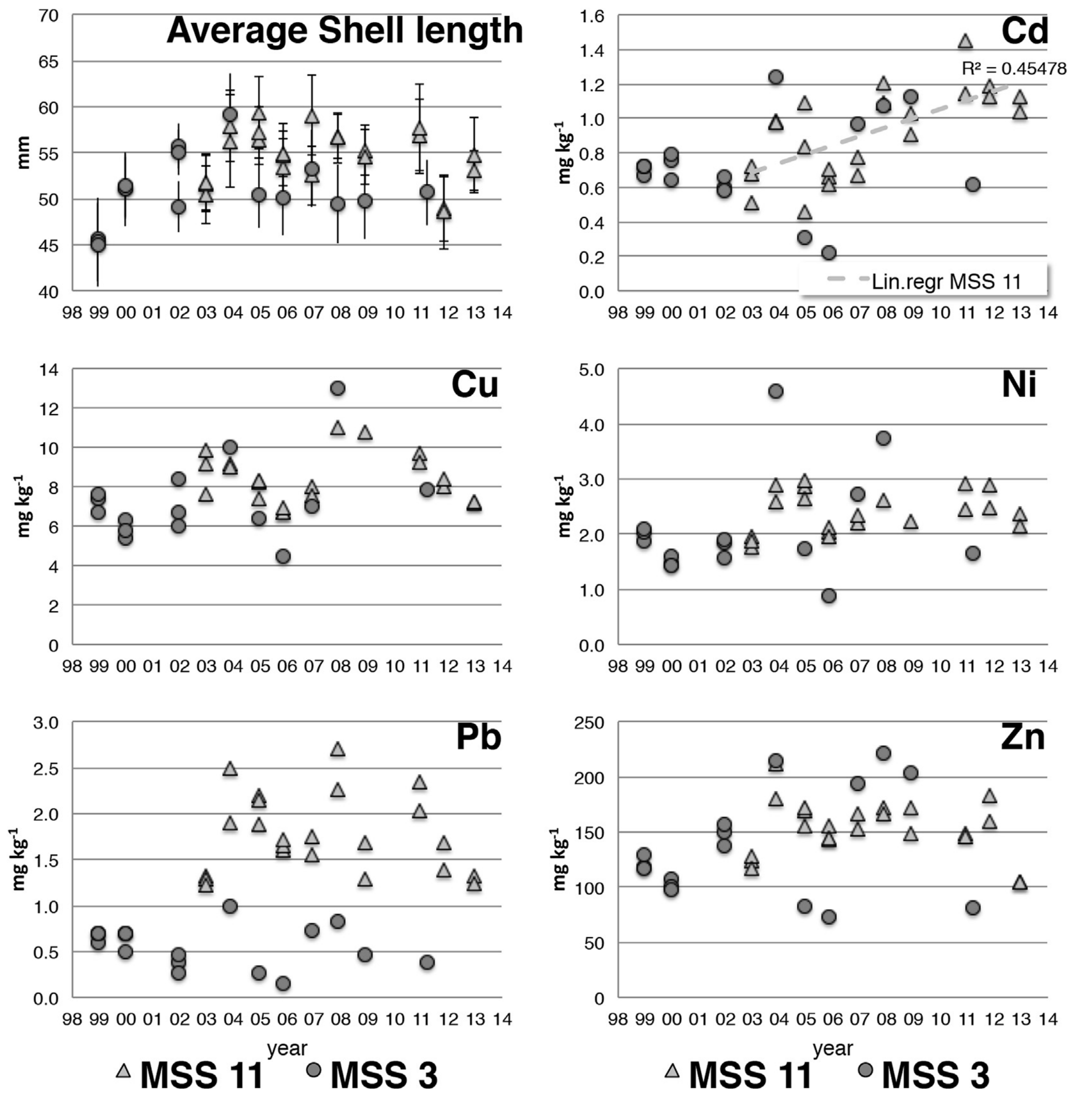

Fig. 7. Time trends from 1999 to 2013 for two Limfjord stations MSS 3 (Nibe Bredning, circle) and MSS 11 (Langerak, triangle). The average shell length with the standard deviation is indicated (n = 25 to 100 for each sample) with up to three replicates per year. Only Cd at MSS 11 was showing significant increasing trend, linear correlation indicated. For 2009, results for Cu, Ni at MSS 3 have been discarded due to expected contamination of samples during homogenisation.

porosity, stability and grain size, water depth and the degree of exposure to wind and waves (Eggleton and Thomas 2004; Atkinson et al. 2007). Physical disturbance of sediments is an important driver, since it was shown to release metals more rapidly than bioturbation or changes in oxygen and salinity (Atkinson et al. 2007). Shallow areas with low tides such as the Limfjorden are likely to be sensitive to increased wind stress due to climate change affecting the metal pollution (Petersen et al., 2013). In addition, increased precipitation and fresh-water inflow is likely to influence metal exposure in the Limfjorden due to its large catchment area with many fresh-water sources.

The climate change scenario with higher temperatures predicted an increase in growth rate, biomass and uptake of metals by mussels (Fig. 6). The resulting changes in body burdens were positive, except for Ni (Fig. 6). In the case of Ni, growth and, hence, biomass increased more than metal uptake, and the body burden was diluted at higher temperatures. A review study found that temperature generally increased the uptake and bioaccumulation of metals, whereas the metal elimination was less affected in aquatic ectotherms (Sokolova and Lannig 2008). It is also possible that elevated temperatures increase the sensitivity to metals or redistribute the metals between bioavailable and non-bioavailable pools, thereby changing the toxicity of the metals (Rainbow 2007; Sokolova and Lannig 2008). Even though the body burdens would increase by 50–116% due to the combination of higher temperatures and higher sea water metal concentrations according to the model scenarios, the resulting metal content was still >4 times lower than the SFT critical pollution level and not of any likely risk for the consumer at the present relatively unpolluted study sites. At other, more polluted sites, elevated temperatures and 50% higher metal concentrations could, on the other hand, have a severe impact on the quality of mussels. The present model may be applied to other locations to predict the outcome of future scenarios of climatic change and its interaction with other, more localized stressors such as metals that, to some extent, can be managed.

4.3. Model uncertainties

The DEB model describes ingestion and growth of blue mussels and was successfully applied to the two study sites (Table A2) with similar temperature regimes, but with significantly different Chl *a*

values (Fig. 3). The DEB model provided detailed information on uptake, growth and spawning that was used in the bio-accumulation model. The performance of the bioaccumulation model was within the ± 2 deviations from the 1:1 ratio in the comparison with observations (Fig. 5), which is similar to previous model evaluations (Luoma and Rainbow 2005; Bourgeault et al. 2011). Some of the model deviations can be explained by the relatively few measurements of ambient metal concentrations that were interpolated over time without capturing the small-scale variability (Fig. 4). A previous study found that the DEB model was sensitive to the frequency of input data (e.g. temperature), affecting the model skills (Montalto et al. 2014).

The predominant route for bioaccumulation is believed to be the dissolved phase for Cd (Wang et al. 1996), through food ingestion for Cu and Ni (Bourgeault et al. 2011) and from both routes for Pb and Zn (Wang et al. 1996; Fisher et al. 1996), although this may vary with salinity, the relative distribution of dissolved and particular phases and their assimilation efficiency (Wang et al. 1996). Our model estimates of AE_m were an average estimate for dissolved and particular phases. The values were, in general, lower than the reported values for the particular phase and higher than those reported for the dissolved phase based on laboratory experiments (Table 1). The relative distribution of the two phases showed a high variability during the spring bloom at site M1 with a high fraction (48–91%) bound to particles. There may be a seasonal pattern in this distribution that could influence AE_m, but there were insufficient data to integrate this into the model. In comparison with data for the particular phase, another explanation for the present lower AE_m is that the bioavailability of native metals are believed to be lower than freshly spiked metals used in laboratory experiments (Wang et al. 2002; Luoma and Rainbow 2005). A previous optimised bioaccumulation model also found at least three times smaller assimilation efficiencies than for laboratory-determined values (Bourgeault et al. 2011). The assimilation efficiency was recommended to be determined for each study site and metal using a combination of field data and model optimisation (Bourgeault et al. 2011). The depuration rates K_e were, on the contrary, assumed to be more robust than AE_m and previously shown to vary little with environmental conditions (Roditi and Fisher 1999; Sokolova and Lannig 2008). Thus, this study supports previous findings that the bioaccumulation model mainly needs recalibration of the assimilation efficiency for new study sites.

5. Conclusion

The body burdens of the metals Cd, Cu, Ni, Pb and Zn in suspended mussels and benthic mussels in the farm area were below the safety limits according to the EU Directives and SFT classification and, hence, suitable to be used for animal feed and food production. However, the GES targets for Cd, Ni and Pb should be reduced in order to protect the mussel production. The model scenarios showed that climate change may increase the metal contamination of mussels, but not to any critical level at the present relatively unpolluted study sites. Farming of suspended mussels is likely to be expanded in Denmark and other areas of the Baltic Sea in order to generate high protein products or as nutrient removers (Gren et al. 2009; Lindahl and Kollberg 2009; Petersen et al. 2014). The expected water metal levels in Danish estuaries are typically close to BAC and far below the GES, particularly for Pb. Only in sites close to harbours or metal industries and river outlets the water concentrations could reach metal levels that would cause concern in mussels farmed for human consumption or feed for husbandry. Such sites would anyway be prone to other types of contamination, both microbiological and from other hazardous substances and, as such, not generally suitable for mussel farming. The model could, however, be applied to other potential sites or scenarios (e.g. climate change) to elucidate any potential effects and hence make recommendations to underpin management strategies in coastal areas, particularly in relation to aquaculture.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ecss.2015.10.010.

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